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## 213.3

LOCAL REGULATION OF ASTROGLIAL PROLIFERATION THROUGH SPECIFIC CELL-CELL INTERACTIONS. Yuji Nakatsui\* and Robert H. Miller. Department of Neurosci. Case Western Reserve Univ. School of Medicine, Cleveland OH 44106

During development and maturation of the vertebrate CNS, proliferation and survival of distinct populations of neuron and glial precursors is closely regulated. The mechanisms that regulate the proliferation of astroglial precursors are not clearly understood. Using purified populations of neural cells *in vitro*, we have investigated the growth factor requirements for proliferation and the effects of glial/glia and neuron/glia interactions on the proliferation of type-1 astrocytes and their precursors. We show that type-1 astrocytes and its precursors proliferate in response to serum factors, epidermal growth factor and fibroblast growth factor (FGF). Serum driven proliferation of type-1 astrocytes was specifically inhibited by density dependent contact inhibition but unaffected by soluble factors from high density astrocyte cultures. The inhibition of cell proliferation was cells type specific. Co-culture of type-1 astrocytes with smooth muscle cells had no effect on their proliferation. Similarly, the proliferation of O-2A progenitor cells was not inhibited by type-1 astrocytes. In co-cultures of type-1 astrocytes and neurons, local interactions between neurons and astrocytes altered the morphology of the glial cells and promoted their proliferation. Since during development, the majority of astrocyte proliferation occurs after the majority of neurons are born. It may be that the primary inhibitor of astroglial proliferation in the mature CNS is mediated through contact between adjacent astrocytes. Such inhibition is locally lost in pathological conditions such as CNS injury leading to astrocyte proliferation. (Supported by NIH grant 25597)

## 213.5

NEURONAL INDUCTION OF EMBRYONIC STEM CELLS IN SERUM-FREE MEDIUM. M.F.A. Finley\*, S. Devata, and J.E. Huettnner. Department of Cell Biology & Physiology, Washington University, St. Louis, MO 63110

Following aggregation and treatment with retinoic acid (RA), embryonic stem (ES) cells differentiate into neurons and glia. Previous work has shown that a variety of cell phenotypes are produced, including both excitatory (glutamatergic), and inhibitory (glycinergic and GABAergic) neurons. In order to learn more about the control of neuronal development, we have begun to study the effect of a variety of growth and differentiation factors on the mature phenotype of ES-derived neurons.

As a prerequisite for this work we have developed appropriate conditions for the efficient induction of neurons in serum-free, defined medium. Initial tests compared Opti-MEM and Neurobasal Medium + B27 supplements (NB + B27) to our standard induction medium: DMEM with nucleosides and 20% calf serum. ES cells survived poorly in Opti-MEM and failed to differentiate. In contrast, ES cells aggregated for 4 days without RA and 4 days with RA (4- / 4+) in NB + B27 yielded a similar proportion of neurons as in complete DMEM (about 40%). Compared to complete DMEM, however, there were roughly half as many cells in total at the end of the induction period in NB + B27.

In the absence of RA, ES aggregates maintained for 8 days in complete DMEM failed to produce neurons; instead, they gave rise to a significant proportion of contractile cells. In contrast, aggregates maintained for 8 days in NB + B27 without RA yielded a small percentage of neurons but no contractile elements. For both 4- / 4+ and 4- / 4+ protocols in NB + B27, the addition of 5 ng/ml bFGF and / or TGF $\alpha$  had little effect on either the proportion or total number of neurons obtained.

NIH NS30888; M.F.A.F. holds a Predoctoral Fellowship from the NSF

## 213.7

NON-VIRALLY MEDIATED GENE TRANSFER INTO HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS. A.L. Vescovi\*, L. Conti\*, P. Frölichsthal, A. Gritti, S. Govoni\*, and E. Cattaneo\*. National Neurological Institute "C.Besta", via Celoria 11, 5 Inst. of Pharmacological Sciences, Univ. of Milan, Via Balzaretti 9, 20133 Milan, and 6 Institute of Pharmacology, Univ. of Pavia, Italy.

The introduction of foreign genes into cells of the central nervous system (CNS) is a method of fundamental relevance for studying the events occurring in the brain under physiological or pathological conditions. Thus far, the use of such a technique to study the basic events underlying the proliferation, fate commitment and differentiation of human CNS precursor cells has been hampered by the lack of both a suitable source of human multipotential CNS cells and of a reliable method to deliver genes into these specific cell type. We report here the successful delivery of foreign genes into multipotential human CNS stem cells. By means of lipofectamine-based transfection the bacterial  $\beta$ -galactosidase or the temperature sensitive allele of the SV40 Large T antigen gene were delivered into foetal (10.5 weeks post-conception) forebrain stem cells that had been grown and expanded for over one year *in vitro*. A transfection efficiency of 7.4% was achieved and expression of the foreign genes was detected in both undifferentiated cells and differentiated neuronal and glial cells. Thus, our data show the possibility of targeting exogenous genes to undifferentiated human CNS stem cells and to their progeny, and define an efficient transfer approach alternative to viral-based methodologies. Supported by the Alzheimer's Association/The Hearst Corporation Pilot Research Grant 94-057 to E.C. and by the Ministry of Health, Italy (ICS49.2/RS93.42) to A.L.V.

## 213.4

ONTOGENY OF CYSTEINE STRING PROTEINS IN PRENATAL RAT BRAIN AND STEM CELL CULTURES.

M.L. Cordeiro<sup>1</sup>, S.D. Zurcher<sup>1</sup>, A. Mastrogiacomo<sup>1</sup>, C. Gundersen<sup>1\*</sup>, and H.L. Kornblum<sup>1,2</sup>. Depts. of Molecular and Medical Pharmacology<sup>1</sup> and Pediatrics<sup>2</sup>, UCLA School of Medicine, Los Angeles, CA 90024.

Cysteine string proteins (CSPs) are intrinsic components of synaptic vesicle membranes. These relatively low mass proteins appear to be involved in the interaction of docked synaptic vesicles with presynaptic calcium ion channels. Recently, CSP immunoreactivity was shown to be abundant in synapse rich areas of the adult rat brain. However, little information is available concerning the role of CSPs in developing brain. We therefore have examined the ontogeny of CSP mRNA in embryonic rat brain (ages E11-P0) using *in situ* hybridization with <sup>35</sup>S-labeled CSP cDNA. By E11, CSP mRNA was relatively homogeneously distributed throughout the CNS. This pattern persisted through E15. By E18, hybridization could be seen predominantly in gray matter and germinal zones, with little expression in white matter. This pattern became even more prominent by the day of birth. Particularly high levels of hybridization were present in the developing cortical plate and hippocampus at these later stages of embryonic development. Because CSP expression was observed in germinal epithelia, we investigated CSP expression in neural stem cells generated from embryonic rat (E17-E19) basal ganglia or cerebral cortex in the presence of TGF $\alpha$ . Western blot identified CSPs in these stem cell cultures. These results suggest that CSPs may have an important role in early development of the CNS.

(Supported by the UCLA Dept. of Molecular and Medical Pharmacology, Dana Foundation, and NIH).

## 213.6

CULTURED SPHERES OF CNS PROGENITOR CELLS FROM THE E13 RAT STRIATUM EXPRESS MEMBERS OF THE JAK-STAT FAMILIES.

C. De Frui<sup>1</sup>, L. Conti<sup>1</sup>, A. Vescovi<sup>2</sup>, M. Mura<sup>2\*</sup>, S. Govoni<sup>3</sup> and E. Cattaneo<sup>1</sup>

<sup>1</sup>Inst. of Pharmacol. Sciences, Univ. of Milano, Via Balzaretti 9, Milano; <sup>2</sup>Besta National Neurological Institute, MI; <sup>3</sup>Inst. of Pharmacology, Univ. of Pavia, Pavia-IT

Stem/progenitor cells from the CNS of different mammalian species have been isolated by different laboratories, expanded for several periods in culture and their differentiation potential analyzed. We started from very early rat striatal material (E13) which had been mechanically dissociated and plated at different densities onto untreated culture dishes. Cells were exposed to EGF or bFGF, alone or in combination, in the absence of a coating substrate. Rat cells detached from the plate within 1-3 days from plating and floated in suspension. Round aggregates of cells (spheres) were soon becoming visible. By 6-7 DIV their size had highly increased. After this period single spheres were dissociated and cells plated at very low density in growth factor enriched medium. Single cells could then be identified which were capable of generating secondary clones *in vitro*. Several of these subclones were grown separately and expanded for over two months. It has been shown that similarly derived spheres contain cells that can differentiate into both neurons and glia. By growing these cells we obtained enough material to perform biochemical analyses. We were interested in whether these proliferating CNS progenitor cells expressed members of the JAK-STAT families of proteins, necessary to transduce from the non tyrosine kinase receptors. Western blot analyses and immunoprecipitation studies were performed on lysates obtained from these expanded CNS progenitor cells. Our data show that JAK2 was abundant in these cells. Furthermore, specific expression of some of the Stat molecules has been identified. Selective recruitment of members of the JAK-STAT by specific stimuli may elicit important biological functions in CNS cells. (Funded by Natl. Res. Coun. Italy)

## 213.8

DIFFERENTIATION OF EGF-RESPONSIVE STEM CELL PROGENY: A TIME COURSE ANALYSIS UNDER VARIOUS CULTURE CONDITIONS. A. Gritti, M. Ferrario, L. Cova, R. Galli, E. Parati and A.L. Vescovi. Natl. Neurol. Inst. "C.Besta", Via Celoria 11, 20133 Milan, Italy.

The establishment of a culture technique enabling the continuous and expansive growth of multipotential CNS stem cells, in the presence of EGF, has raised the intriguing possibility of preparing mixed neuronal/ glial cultures avoiding the repeated use of primary brain tissue. Unfortunately, cultures from EGF-generated cells that are induced to differentiate by removal of EGF contain only a small percentage of neuronal cells, as compared to those obtained using traditional techniques. In order to clarify this phenomenon, we studied the time course of expression of antigenic markers specific for neuronal, astroglial or oligodendroglial cells, in cultures of EGF-generated cells differentiating under various conditions. We found that the percentage of neuronal cells increased at day 2, 4 and 6 after EGF removal, reaching a maximum of 10-12% at this later time point. The number of neurons then dropped over time, reaching a minimum (3-5%) at 12 days, regardless of the presence or absence of serum. Conversely, astrocytes were barely detectable at day 2 but their number increased overtime reaching a maximum at 12 days. The presence of 2% serum strongly affected the percentage of astrocytes in the culture. Serum-containing cultures embodied 15-25% more astrocytes (reaching a maximum of 60-70% at 12 days) than serum-free cultures. Our data indicate that culture conditions can strongly affect the differentiation of stem cell progeny and show that a great deal of death occurs in these cultures during early stages of neuronal differentiation. Further investigations are currently underway to confirm preliminary results showing that FGF-2 may antagonise neuronal death in this system. Supported by The Ministry of Health, Italy (ICS49.2/RS93.42).

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